Claims

1. A method for measuring α-amylase activity in a sample, comprising

forming a reaction mixture by contacting a sample with a detectably labeled starch substrate for a time sufficient for α-amylase in the sample to hydrolyze the starch substrate, thereby releasing soluble detectably labeled starch fragments,

separating the soluble detectably labeled starch fragments from the reaction mixture, and

determining the level of hydrolysis of the detectably labeled starch substrate as a measurement of α-amylase activity in the sample.

2. The method of claim 1, wherein the sample is selected from the group consisting of a flour sample, a stock sample, and an amylase concentrate sample.

3. The method of claim 1, wherein determining the level of hydrolysis of the detectably labeled starch substrate comprises quantifying the detectably labeled starch substrate.

4. The method of claim 1, wherein determining the level of hydrolysis of the detectably labeled starch substrate comprises quantifying the soluble detectably labeled starch substrate fragments.

5. The method of claim 1, further comprising calculating the α-amylase activity in the sample by correlating the quantity of detectably labeled starch to an α-amylase standard.

6. The method of claim 1, further comprising calculating the α-amylase activity in the sample by correlating the quantity of soluble detectably labeled starch fragments to an α-amylase standard.

7. The method of claim 1, wherein the detectably labeled starch substrate is potato starch.

8. The method of claim 1, wherein the detectably labeled starch substrate comprises D-glucose residues and is labeled on about one of every 300-1300 D-glucose residues of the starch substrate.

9. The method of claim 1, wherein the starch substrate is detectably labeled with a label compound selected from the group consisting of fluorescent, enzyme, radioactive, metallic, biotin, chemiluminescent, and bioluminescent molecules.

10. The method of claim 9, wherein the label is a fluorophore.

11. The method of claim 10, wherein the fluorophore is selected from the group consisting of 5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid (EDANS), fluorescein isothiocyanate (FITC), and Marina Blue.

12. The method of claim 1, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises filtering the reaction mixture to remove from the mixture detectably labeled starch substrate.

13. The method of claim 12, wherein the step of filtering includes the addition of a filtration aid selected from the group consisting of resin, glass beads, beads, and celite.

14. The method of claim 1, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises centrifuging the reaction mixture to remove from the mixture detectably labeled starch substrate.

15. The method of claim 14, further comprising measuring an aliquot of the supernatant of the centrifuged reaction mixture.

16. The method of claim 1, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises obtaining an aliquot of

the reaction mixture and centrifuging the aliquot of the reaction mixture to remove from the aliquot detectably labeled starch substrate.

17. The method of claim 1, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises contacting the fragments with an agent that binds to the detectably labeled starch fragments.

18. The method of claim 17, wherein the agent is a lectin.

19. The method of claim 17, wherein the agent is an antibody.

20. The method of claim 1, wherein the sample is an aqueous slurry.

21. The method of claim 1, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about one minute.

22. The method of claim 1, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about five minutes.

23. The method of claim 1, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about ten minutes.

24. The method of claim 1, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about fifteen minutes.

25. A method for measuring α-amylase activity in a sample, comprising

forming a reaction mixture by contacting a sample with a detectably labeled starch substrate attached to a surface, for a time sufficient for α-amylase in the sample to hydrolyze the starch substrate, thereby releasing soluble detectably labeled starch fragments,

separating the soluble detectably labeled starch fragments from the reaction mixture, and

determining the level of hydrolysis of the detectably labeled starch substrate as a measurement of α-amylase activity in the sample.

26. The method of claim 25, wherein the sample is selected from the group consisting of a flour sample, a stock sample, and an amylase concentrate sample.

27. The method of claim 25, wherein the surface is selected from the group consisting of a tube, a centrifuge tube, a cuvette, a dipstick, a multiwell plate, a slide, a coverslip, a card, a bead, and a plate.

28. The method of claim 25, wherein determining the level of hydrolysis of the detectably labeled starch substrate comprises quantifying the soluble detectably labeled starch substrate fragments.

29. The method of claim 28, further comprising calculating the α-amylase activity in the sample by correlating the quantity of soluble detectably labeled starch fragments to an α-amylase standard.

30. The method of claim 25, wherein determining the level of hydrolysis of the detectably labeled starch substrate comprises quantifying the detectably labeled starch substrate after separating the soluble detectably labeled starch fragments from the reaction mixture.

31. The method of claim 25, further comprising, releasing the detectably labeled starch substrate from the surface after separating the soluble detectably labeled starch fragments from the reaction mixture.

32. The method of claim 31, wherein determining the level of hydrolysis of the detectably labeled starch substrate comprises quantifying the detectably labeled starch substrate after releasing the detectably labeled starch substrate from the surface.

33. The method of claim 25, further comprising calculating the α-amylase activity in the sample by correlating the quantity of detectably labeled starch to an α-amylase standard.

34. The method of claim 25, wherein the detectably labeled starch substrate is potato starch.

35. The method of claim 25, wherein the detectably labeled starch substrate comprises D-glucose residues and is labeled on about one of every 300-1300 D-glucose residues of the starch substrate.

36. The method of claim 25, wherein the starch substrate is detectably labeled with a label compound selected from the group consisting of fluorescent, enzyme, radioactive, metallic, biotin, chemiluminescent, and bioluminescent molecules.

37. The method of claim 36, wherein the label is a fluorophore.

38. The method of claim 37, wherein the fluorophore is selected from the group consisting of 5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid (EDANS), fluorescein isothiocyanate (FITC), and Marina Blue.

39. The method of claim 25, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises filtering the reaction mixture to remove from the mixture detectably labeled starch substrate.

40. The method of claim 39, wherein the step of filtering includes the addition of a filtration aid selected from the group consisting of resin, glass beads, beads, and celite.

41. The method of claim 25, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises centrifuging the reaction mixture to remove from the mixture detectably labeled starch substrate.

42. The method of claim 41, further comprising measuring an aliquot of the supernatant of the centrifuged reaction mixture.

43. The method of claim 25, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises obtaining an aliquot of the reaction mixture and centrifuging the aliquot of the reaction mixture to remove from the aliquot detectably labeled starch substrate.

44. The method of claim 25, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises contacting the fragments with an agent that binds to the detectably labeled starch fragments.

45. The method of claim 44, wherein the agent is a lectin.

46. The method of claim 44, wherein the agent is an antibody.

47. The method of claim 25, wherein the sample is an aqueous slurry.

48. The method of claim 25, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about one minute.

49. The method of claim 25, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about five minutes.

50. The method of claim 25, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about ten minutes.

51. The method of claim 25, wherein the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about fifteen minutes.

52. A kit for measuring α-amylase activity in a sample, comprising

a first container containing a detectably labeled starch substrate, a second container containing an α-amylase standard, and instructions for measuring the α-amylase activity in the sample.

53. The kit of claim 52, wherein the sample is selected from the group consisting of a flour sample, a stock sample, and an amylase concentrate sample.

54. The kit of claim 52, wherein the detectably labeled starch substrate is potato starch.

55. The kit of claim 52, wherein the detectably labeled starch substrate comprises D-glucose residues and is labeled on about one of every 300-1300 D-glucose residues of the starch substrate.

56. The kit of claim 52, wherein the starch substrate is detectably labeled with a label compound selected from the group consisting of fluorescent, enzyme, radioactive, metallic, biotin, chemiluminescent, and bioluminescent molecules.

57. The kit of claim 56, wherein the label is a fluorophore.

58. The kit of claim 57, wherein the fluorophore is selected from the group consisting of 5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid (EDANS), fluorescein isothiocyanate (FITC), and Marina Blue.

59. The kit of claim 57, wherein the instructions for measuring the α-amylase activity in a sample recite a method comprising forming a reaction mixture by contacting a sample with a detectably labeled starch substrate for a time sufficient for α-amylase in the sample to hydrolyze the starch substrate, thereby releasing soluble detectably labeled starch fragments, separating the soluble detectably labeled starch fragments from the reaction mixture, and quantifying the soluble detectably labeled starch as a measurement of α-amylase activity in the sample.

60. The kit of claim 59, wherein the instructions further recite calculating the α-amylase activity in the sample by correlating the quantity of soluble detectably labeled starch fragments to an α-amylase standard.

61. The kit of claim 59, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises filtering the reaction mixture to remove from the mixture detectably labeled starch substrate.

62. The kit of claim 61, wherein the step of filtering includes the addition of a filtration aid selected from the group consisting of resin, glass beads, beads, and celite.

63. The kit of claim 59, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises centrifuging the reaction mixture to remove from the mixture detectably labeled starch substrate.

64. The kit of claim 63, further comprising measuring an aliquot of the supernatant of the centrifuged reaction mixture.

65. The kit of claim 59, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises obtaining an aliquot of the reaction mixture and centrifuging the aliquot of the reaction mixture to remove from the aliquot detectably labeled starch substrate.

66. The kit of claim 59, wherein the step of separating the soluble detectably labeled starch fragments from the reaction mixture comprises contacting the fragments with an agent that binds to the detectably labeled starch fragments.

67. The kit of claim 66, wherein the agent is a lectin.

68. The kit of claim 66, wherein the agent is an antibody.

69. The kit of claim 59, wherein the instructions further recite that the sample is an aqueous slurry.

70. The kit of claim 59, wherein the instructions further recite that the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about one minute.

71. The kit of claim 59, wherein the instructions further recite that the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about five minutes.

72. The kit of claim 59, wherein the instructions further recite that the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about ten minutes.

73. The kit of claim 59, wherein the instructions further recite that the sample is contacted with the detectably labeled starch substrate for a reaction time of at least about fifteen minutes.

74. A method of determining amylase in a sample comprising:
placing about 6ml incubation buffer in a substrate tube,
warming the substrate tube to 45°C,
adding about 200 mg of the sample to the warmed substrate tube,
incubating the sample mixture in the substrate tube 10 min at 45°C,
adding about 4 ml stop buffer to the sample mixture in the substrate tube,
filtering the stopped sample mixture into a container,
determining the fluorescence in the filtrate, and
optionally converting the fluorescence value into a Falling Number Equivalent value.

75. The method of claim 74, wherein the container is a cuvette.

76. The method of claim 74, wherein the fluorescence is determined in a fluorometer.

77. The method of claim 74, wherein the amylase comprises one or more amylases selected from the group consisting of cereal amylase, bacterial amylase, and fungal amylase.

78. The method of claim 74, wherein the sample selected from the group consisting of a flour sample, a stock sample, and an amylase concentrate sample.

79. The method of claim 74, wherein the filtering is filtering through a microfiber filter.

80. A method of determining amylase in a sample comprising:

placing an about 3g sample into a first container,

adding a sufficient amount of fungal incubation buffer to have the total weight of sample plus buffer equal of about 30g,

mixing the solution,

extracting the solution for 5 minutes at 45°C,

adding the about 8ml of the extract to a substrate tube,

incubating extract in substrate tube 10 minutes at 45°C,

adding about 2ml stop buffer the tube,

mixing the contents of the tube,

filtering the mixture into a second container,

determining the fluorescence in the filtrate, and

optionally converting the fluorescence value into an Enzyme Units Equivalent value.

81. The method of claim 80, wherein the first container is a tube.

82. The method of claim 80, wherein the second container is a cuvette.

83. The method of claim 80, wherein the fluorescence is determined in a fluorometer.

84. The method of claim 80, wherein the sample comprises one or more amylases selected from the group consisting of cereal amylase, bacterial amylase, and fungal amylase.

85. The method of claim 80, wherein the sample is selected from the group consisting of a flour sample, a stock sample, and an amylase concentrate sample.

86. The method of claim 85, wherein the flour sample is a wheat flour sample.

87. The method of claim 80, wherein the filtering is filtering through a microfiber filter.

88. A method of determining amylase in a sample comprising:
placing about 200mg of the sample into a container,
adding about 20ml fungal incubation buffer to the sample,
mixing the sample solution,
diluting about 2ml of the solution with 10ml incubation buffer in a container,
optionally further diluting the diluted solution to obtain a concentration within range of about 0.1-1.0 SKB unit/ml,
placing about 8ml of the diluted sample into a container,
incubating the about 8ml diluted sample 10 minutes at 45°C,
adding about 2ml stop buffer to the 8ml diluted sample,
filtering the mixture through a filter into a detection container,
determining the fluorescence in the filtrate, and
optionally converting the fluorescence value into an Enzyme Units Equivalent value and multiplying by the dilution factor as a measure of the original amylase concentration.

89. The method of claim 88, wherein the container is a tube.

90. The method of claim 88, wherein the detection container is a cuvette.

91. The method of claim 88, wherein the fluorescence is determined in a fluorometer.

92. The method of claim 88, wherein the sample comprises one or more amylases selected from the group consisting of cereal amylase, bacterial amylase and fungal amylase.

93. The method of claim 88, wherein the sample is selected from the group consisting of a flour sample, a stock sample, and an amylase concentrate sample.

94. The method of claim 93, wherein the flour sample is a wheat flour sample.

95. The method of claim 88, wherein the filtering is filtering through a microfiber filter.